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(71) Applicant: THE UNIVERSITY OF TENNESSEE RESEARCH CORPORATION [US/US]; Suite 403, 1534 White Avenue, Knoxville, TN 37996–1527 (US).

(72) Inventors: SOLOMON, Alan; 2705 Riverside Drive, Knoxville, TN 37914 (US). HRNCIC, Rudi; 1041 Venice Road, Knoxville, TN 37923 (US). WALL, Jonathan, S.; 808 Normandy Drive, Knoxville, TN 37919 (US).

(74) Agent: ADLER, Reid, G.; Morgan, Lewis & Bockius LLP, 1800 M Street, N.W., Washington, DC 20036–5869 (US).

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(54) Title: METHODS FOR AMYLOID REMOVAL USING ANTI-AMYLOID ANTIBODIES

(57) Abstract

Methods and related immunoglobulin peptides and fragments thereof are disclosed that enhance the cell-mediated immune response of a patient to deposits of amyloid fibrils. These methods exploit the opsonizing effect of antibodies directed toward amyloid material or its component parts.

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fibrils in conditions modulated by amyloid fibrils without such a therapeutic intervention, presumably because the amyloid fibrils are themselves relatively non-immunogenic.

To treat a patient with amyloidosis, a therapeutically effective dose of immunoglobulin polypeptide or fragment thereof according to the present invention is administered together with a pharmaceutically suitable carrier or excipient. Upon the binding or adhering of such immunoglobulin polypeptides to undesired deposits of amyloid fibrils, the latter are believed to be opsonized.

Single or multiple administrations of the compositions of the present invention can be carried out in dosages and by administration protocols known to those skilled in the art for the administration of other therapeutic antibody products. These parameters may be selected and/or optimized by the physician treating a particular patient.

Preferably, a therapeutically effective dose of a pharmaceutical formulation of the present invention should deliver a quantity of anti-amyloid immunoglobulin polypeptide sufficient to substantially inhibit the undesired deposition of amyloid fibrils or to substantially inhibit the rate of any undesired deposition of amyloid fibrils. More preferably, the formulations should reduce the overall burden of deposited amyloid fibrils in a patient. Further, administration of such formulations should begin shortly after diagnosis of amyloidosis and continue until symptoms are substantially abated and for a period thereafter. In well established cases of disease, loading doses followed by maintenance doses may be required.

Definitions

The terms "peptide," "polypeptide" or "protein" are used interchangeably herein. The term "substantial identity," when referring to polypeptides, indicates that the polypeptide or protein in question is at least about 30% identical to an entire naturally occurring protein or a portion thereof, usually at least about 70% identical, and preferably at least about 95% identical.

As used herein, the terms "isolated," "substantially pure" and "substantially homogenous" are used interchangeably and describe a protein that has been separated from components which naturally accompany it. A substantially purified protein will

system, including mice, rats, rabbits, human cell lines, or other vertebrates capable of producing antibodies by well-known methods. Variable regions or CDRs may be produced synthetically, by standard recombinant methods, including polymerase chain reaction ("PER") or through phage-display libraries. For phage display methods, see for example, McCafferty *et al.*, Nature 348:552-554 (1990); Clackson *et al.*, Nature 352:624-628 and Marks *et al.*, Biotechnology 11:1145- 1149 (1993). Suitable prokaryotic systems such as bacteria, yeast and phage may be employed.

Suitable source cells for the DNA sequences and host cells for immunoglobulin expression and secretion can be obtained from a number of sources, such as the American Type Culture Collection ("Catalogue of Cell Lines and Hybridomas," Fifth edition (1985) Rockville, Maryland, U.S.A., which is incorporated herein by reference).

In addition to the chimeric and "humanized" immunoglobulins specifically described herein, other substantially identical modified immunoglobulins can be readily designed and manufactured utilizing various recombinant DNA techniques well known to those skilled in the art. In general, modifications of the genes may be readily accomplished by a variety of well-known techniques, such as PCR and site-directed mutagenesis (see, Gillman and Smith, Gene 8:81-97 (1979) and S. Roberts *et al.*, Nature 328:731-734 (1987), both of which are incorporated herein by reference).

Alternatively, polypeptide fragments comprising only a portion of the primary immunoglobulin structure may be produced. For example, it may be desirable to produce immunoglobulin polypeptide fragments that possess one or more immunoglobulin activities in addition to, or other than, antigen recognition (*e.g.*, complement fixation).

Immunoglobulin genes, in whole or in part, may also be combined with functional regions from other genes (*e.g.*, enzymes), or with other molecules such as toxins, labels and targeting moieties to produce fusion proteins (*e.g.*, "immunotoxins") having novel properties. In these cases of gene fusion, the two components are present within the same polypeptide chain. Alternatively, the immunoglobulin or fragment thereof may be chemically bonded to the toxin or label by any of a variety of well-known chemical procedures. For example, when the label or cytotoxic agent is a protein and the

then raised against the synthetic fibrils using standard immunization techniques, typically in mice or rabbits. Monoclonal cell lines secreting anit-fibril antibodies are produced using standard hybridoma techniques.

The anti-amyloid immunoglobulin polypeptides of the invention may be prepared by any of a number of well-known techniques. For instance, they may be prepared by immunizing an animal with purified or partially purified human amyloid. The animals immunized can be any one of a variety of species which are capable of immunologically recognizing epitopes characteristic of the human type amyloid extracellular domain, such as murine, lagomorph, equine, etc.

Monoclonal antibodies of the invention may be prepared by immortalizing cells comprising nucleic acid sequences which encode immunoglobulin polypeptides or portions thereof that bind specifically to antigenic determinants characteristic of the extracellular domain of the human type amyloid. The immortalization process can be carried out by hybridoma fusion techniques, by viral transformation of antibody-producing lymphocytes, recombinant DNA techniques, or by techniques that combine cell fusion, viral transformation and/or recombinant DNA methodologies. Immunogens to raise the monoclonal antibodies include synthetic amyloid fibrils as described, for example by, A. Lomakin *et al.*, 1997 Proc. Nat'l Acad. Sci. USA 94: 7942-7, which is incorporated herein by reference.

As the generation of human anti-amyloid monoclonal antibodies may be difficult with conventional immortalization techniques, it may be desirable to first make non-human antibodies and then transfer via recombinant DNA techniques the antigen binding regions of the non-human antibodies, *e.g.*, the Fab, complementarity determining regions (CDRs) or hypervariable regions, to human constant regions (Fc) or framework regions as appropriate to produce substantially human molecules. Such methods are generally known in the art and are described in, for example, U.S. 4,816,397, PCT publication WO 90/07861, and EP publications 173494 and 239400, wherein each is incorporated herein by reference. However, completely human antibodies can be produced in transgenic animals. The desired human immunoglobulin genes or gene segments can be isolated, for example by PCR from human B cells, the DNA cloned into appropriate vectors for expression in eukaryotic cells and the cloned DNA introduced into animals to produce

transgenics. Animals suitable for the production of transgenics expressing human immunoglobulin include mice, rats, rabbits and pigs with rodents of transgenics that express human immunoglobulins should preferably have one or more of their endogenous immunoglobulin loci inactivated or "knocked-out" to facilitate identification and isolation of the human antibodies (See *e.g.*, Lonberg, et al. Nature 368:856-859 (1994)).

The resulting chimeric antibodies or chimeric immunoglobulin polypeptides that bind to human amyloid are also within the scope of the present invention. A typical therapeutic chimeric antibody would be a hybrid protein consisting of the variable (V) or antigen-binding domain from a mouse immunoglobulin specific for a human amyloid antigenic determinant, and the constant (C) or effector domain from a human immunoglobulin, although domains from other mammalian species may be used for both variable and constant domains. As used herein, the therm "chimeric antibody" also refers to antibodies coded for by immunoglobulin genes in which only the CDRs are transferred from the immunoglobulin that specifically recognizes the antigenic determinants, the remainder of the immunoglobulin gene being derived from a human (or other mammalian, as desired) immunoglobulin gene. As discussed before, this type of chimeric antibody is referred to as a "humanized" (in the case of a human immunoglobulin gene being used) antibody. Also considered are recombinant human antibodies that do not contain sequences of another species.

The hypervariable regions of the variable domains of the anti-amyloid immunoglobulin polypeptides comprise a related aspect of the invention. The hypervariable regions, or CDRs, in conjunction with the framework regions (those portions of immunoglobulin light and heavy chain variable regions that are relatively conserved among different immunoglobulins in a single species), enable the anti-amyloid immunoglobulin polypeptides to recognize and thus bind to human amyloid. The hypervariable regions can be cloned and sequenced. Once identified, these regions that confer specific recognition of human amyloid can then be cloned into a vector for expression in a host as part of another immunoglobulin molecule or as a fusion protein, *e.g.*, a carrier molecule which functions to enhance immunogenicity of the cloned idiotype.

The anti-amyloid immunoglobulin polypeptides of the invention will generally be used intact, or as immunogenic fragments, such Fv, Fab, $F(ab')_2$ fragments. The fragments may be obtained from antibodies by conventional techniques, such as by proteolytic digestion of the antibody using, *e.g.*, pepsin, papain or other proteolytic enzymes, or by recombinant DNA techniques in which a gene or portion thereof encoding the desired fragment is cloned or synthesized, and expressed in a variety of hosts.

Those skilled in the art will realize that "anti-idiotypic" antibodies can be produced by using a specific immunoglobulin as an immunogen in accordance with standard techniques. For example, infection or immunization with an amyloid fibril or fragment thereof, induces a neutralizing immunoglobulin, which has on its Fab variable region combining site an image of the amyloid that is unique to that particular immunoglobulin, *i.e.*, an idiotype. Immunization with such an anti-amyloid immunoglobulin induces an anti-idiotype antibody, which has a conformation at its combining site that mimics the structure of the original amyloid antigen. These anti-idiotype antibodies may therefore be used instead of the amyloid antigen. See, for example, Nisonoff (1991) J. Immunol. 147:2429-2438, which is incorporated herein by reference.

The following working examples specifically point out preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure. Other generic configurations will be apparent to one skilled in the art.

Example 1 Unassisted Resolution of Human IgLC Amyloid in Murine Host

Human IgLC amyloid was extracted and purified from infected organs obtained during an autopsy. The first experiments involved transplanting 50-200 mg of this amyloid material into a Balb/c mouse. The amyloid mass, or "amyloidoma," was prepared in sterile PBS by serial sonication and grinding steps in order to produce a fine suspension of amyloid fibrils complete with the accessory molecules found *in vivo*. This procedure was performed to allow the amyloid to be injected into the mice through a wide-gauge hypodermic needle.

The amyloid material, equivalent to 10% of the body weight of the animal, was injected into mice (under anesthetic) between the scapula, which resulted in a large mass being visible (see Figure 1A). The mouse required 15-18 days to achieve the complete removal of the amyloidoma (see Figure 1B), after which the animal appeared healthy and lived a normal life span. The removal of the amyloidoma was determined subjectively by the experimenter; by simply palpating the injection site, an amyloidoma, like a hard pea, can be easily felt under the skin.

Example 2 Involvement of Both Antibody-Mediated and Cellular Immunity in the Removal of Amyloidomas

The involvement of anti-amyloid antibodies in the removal of amyloidomas was shown by screening serum from a mouse previously injected with amyloid material against a sample of the injected material. This was done by Western blot analysis using suitable dilutions of the mouse serum as the primary antibody. It was shown that there were antibodies to every component of the amyloid matrix, *i.e.*, every band on the gel was stained by the mouse serum, even at a 10,000-fold serum dilution (data not shown).

The involvement of a cellular component was demonstrated by *in vitro* neutrophil binding assays (see Figures 2A and 2B) and by using knockout-mutant mouse strains (data not shown). Figures 2A and 2B show human neutrophils adhering to human amyloid after the amyloid was treated with mouse anti-human IgLC mAbs. This shows that the mouse mAb can bind to human amyloid as well as attract human neutrophils.

Studies of knockout-mutant mouse strains further support a finding of antibody involvement in amyloid removal. First, *scid/scid* mice, which lack B and T lymphocytes, were unable to remove an injected amyloidoma even after three months (data not shown). Second, CD18 knockout animals were unable to remove the amyloidoma as rapidly as normal animals. CD18 knockout animals are 97% deficient in CD18, a cell surface integrin found on granulocyte/macrophage lineages. Although these cells cannot leave the circulation, the animals are B and T cell competent and can therefore mount an antibody response. Third, nude mice, which have no white blood cells, were unable to remove the amyloidoma.

Furthermore, amyloid that had been incubated with amyloid-reactive serum from another mouse, when implanted into the second mouse, was removed within 4 days. In this experiment a Balb/c mouse was injected with 50 mg HIG amyloid and left for 1 week, after which it was bled by tail-vein clipping. The blood was spun down at 1500 rpm and the cells removed by aspiration. The plasma was stored at 4 °C until used. Another preparation of HIG amyloid (100 mg) was prepared by suspending in sterile PBS to which was added 1 ml of plasma from the previous mouse. This preparation was then injected into a second mouse (Balb/c) and the amyloid was removed in 4 days. Thus, it was concluded that the process could be sped up by opsonizing the material prior to injection.

Example 3 ELISA Screening of IgLC Subsets

A systematic study was performed using ELISA techniques to screen a large number of human extracted amyloid samples using mAbs raised against the IgLC subsets (λ1, λ2, λ3, λ4, λ5, λ6, κ1, κ2, κ3, κ4, free κ and λ and total κ and λ). Interestingly, it was found that more often than not, the amyloids tested positive with mAbs specific for their own subtype, the total κ or λ antibodies and a κ1(57-18H12), κ4(11-1F4) and λ8(31-8C7) mAb. These latter three reagents were found to react in a non-subgroup specific manner, *i.e.*, κ1 reacted with amyloids comprised of IgLCs other than κ1; and the other two mAbs exhibit the same quality. This shows that the epitope recognized by these antibodies may be a general feature of amyloid fibrils, indicating the possibility of a shared amyloid epitope that can be targeted.

Example 4 Immunochemical Staining

Tissue samples from amyloid patients were stained using standard immunochemical techniques and a similar binding phenomenon was observed. Figures 3A-3D show that anti-κ1 binds to the κ1 amyloid and, surprisingly, that the anti-κ4 reacts with the κ1 amyloid, suggesting an amyloid epitope that these antibodies may recognize. Additionally, the anti-κ4 reacts with λ-containing amyloid (not illustrated). This is an example of cross-isotype reactivity. However, the results from the ELISA and the immunohistochemistry were not always consistent. This is likely due to the inherent

difference in what you are looking at, *i.e.*, ELISA is a liquid phase binding assay using extracted purified amyloid, whereas immunohistochemistry is performed on fixed tissue sections on a slide.

Samples of hybridoma cells that secrete anti-κ1 (57-18-H12 (ATCC Acc. No.__)), anti-κ4 (11-1F4 (ATCC Acc. No.___)) and anti-κ8 (31-8c7 (ATCC Acc. No.___)) monoclonal antibodies were deposited with the American Type Culture Collection (ATCC) on May 21, 1999 in compliance with the Budapest Treaty.

Example 5 *In Vivo* Studies of Anti-IgLC Subgroups

0.1 mg of one of three antibodies -- κ1, κ4, or λ8, identified above -- was injected into the thigh of a mouse into which amyloid had been introduced in the form of an amyloidoma as described above. The κ1 and κ4 reagents resulted in the complete removal by the host of most amyloid fibril species tested within 7 days (as little as 4 days for certain sources of amyloid). Figure 4 shows fluoresceinated κ4 mAb binding to human amyloid.

The λ8 reagent, which is reactive in certain instances in both *in vitro* studies (above), increased the resolution of amyloidomas by up to about 10% in *in vivo* experiments.

Example 6 *In Vivo* Studies of Anti-IgLC Subgroups

Human amyloid was isolated from a patient with inflammation-associated, AA-amyloid and prepared for injection into Balb/C mice by repeated sonication and grinding in order to permit its injection into the mouse (see Example 1). Immediately after the injection of 100 mg of human AA-amyloid extract, the mice were treated with 100 μg of κ4 mAb, anti-AA mAb, no mAb and non-specific control mAb (anti-free κ). Complete resolution of the material was observed with 48 hours in the animals that had been treated with the κ4 and anti-AA mAbs. In contrast, the control animals had a large mass of amyloid remaining at the site of injection.

Example 7 Production of Specific Anti-Amyloid Fibril mAbs

Synthetic amyloid fibrils were prepared *in vitro* and used as an immunogen in mice to produce a first generation of anti-amyloid fibril mAbs. Briefly, recombinant λ6-light chain, variable region peptides were produced, isolated and purified using a bacterial expression system and standard protein purification techniques. Synthetic fibrils were prepared from these peptides by extended periods of agitation in solution as described, for example, in Wall et al., "*In vitro* Immunoglobulin Light Chain Fibrillogenesis," METHODS IN ENZYMOLOGY, Vol. 309 (In Press), which is incorporated herein by reference in its entirety. Fibrils were concentrated by centrifugation at 17,000 × g for 20 minutes at room temperature.

The concentrated fibrils were then used to immunize Balb/c mice over a period of several weeks. Monoclonal cell lines secreting anti-fibril antibodies were produced using standard hybridoma techniques. The resultant antibodies have demonstrable anti-fibril activity based upon ELISA assays, described in Example 3. These antibodies reacted with 99% of all human IgLC amyloid extracts tested to date irrespective of the nature of the isotype or subgroup of the precursor protein when tested by ELISA. Similarly, the antibodies reacted in an ELISA format with isolated murine AA-amyloid and synthetic fibrils composed of a peptide derived from the Alzheimer's protein Aβ [Aβ(25-35)].

It should be understood that the foregoing discussion and examples merely present a detailed description of certain preferred embodiments. It therefore should be apparent to those of ordinary skill in the art that various modifications and equivalents can be made without departing from the spirit and scope of the invention. All references, articles and patents identified above are herein incorporated by reference in their entirety.

What is claimed is:

1. A method of treating a patient having an amyloid deposition disease comprising the step of administering to the patient

a) a therapeutically effective dose of at least one immunoglobulin polypeptide or a fragments thereof, wherein the immunoglobulin polypeptide or fragment thereof binds to an amyloid fibril; and

b) a pharmaceutically acceptable carrier.

2. The method of claim 1, wherein the immunoglobulin polypeptide or fragment thereof is raised against an immunoglobulin light-chain.

3. The method of claim 1, wherein binding of the immunoglobulin polypeptide or fragment thereof opsonizes the amyloid fibril.

4. The method of claim 1, wherein the immunoglobulin polypeptide or fragment thereof is a monoclonal antibody.

5. The method of claim 4, wherein the monoclonal antibody is a humanized antibody.

6. The method of claim 4, wherein the monoclonal antibody is a chimeric antibody.

7. The method of claim 6, wherein the chimeric antibody is a humanized antibody.

8. The method of claim 4, wherein the antibody is a labeled antibody.

9. The method of claim 4, wherein the monoclonal antibody is selected from the group consisting of κ1 (57-18H12), κ4 (11-1F4), λ8 (31-8C7), and combinations thereof.

10. An immunoglobulin polypeptide or fragment thereof that binds to an amyloid fibril and is effective to enhance the cellular immune response of a patient to remove disease-associated amyloid fibril deposits.

11. The immunoglobulin polypeptide or fragment thereof of claim 10, wherein the immunoglobulin polypeptide or fragment thereof is a monoclonal antibody or fragment thereof.

12. The immunoglobulin or fragment thereof of claim 11, wherein the monoclonal antibody is a humanized antibody.

13. The immunoglobulin polypeptide or fragment thereof of claim 11, wherein the monoclonal antibody is a chimeric antibody.

14. The immunoglobulin polypeptide or fragment thereof of claim 13, wherein the chimeric antibody is a humanized antibody.

15. The immunoglobulin polypeptide or fragment thereof of claim 11, wherein the antibody is a labeled antibody.

16. The immunoglobulin polypeptide or fragment thereof of claim 11, wherein the monoclonal antibody is selected from the group consisting of κ1 (57-18H12), κ4 (11-1F4), λ8 (31-8C7), and combinations thereof.

17. The monoclonal antibody or fragment thereof of claim 16, wherein the monoclonal antibody is a humanized antibody.

18. The immunoglobulin polypeptide or fragment thereof of claim 10, wherein the immunoglobulin polypeptide or fragment thereof has been raised against synthetic amyloid fibrils.

19. A pharmaceutical composition comprising the immunoglobulin peptide or fragment thereof of any of claims 10-17.

20. A nucleic acid molecule which encodes a polypeptide comprising at least a hypervariable region of the immunoglobulin polypeptide of any of claims 10-17.

21. A host cell comprising a nucleic acid molecule of claim 20.

22. A method of producing an immunoglobulin polypeptide comprising the step of culturing the host cell of claim 21.

Day 1

A

Day 14

B

Figure 1

A

B

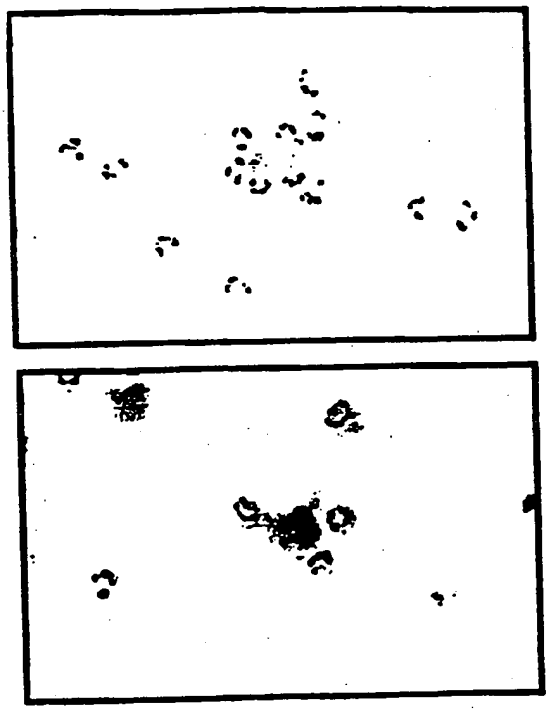

Figure 2

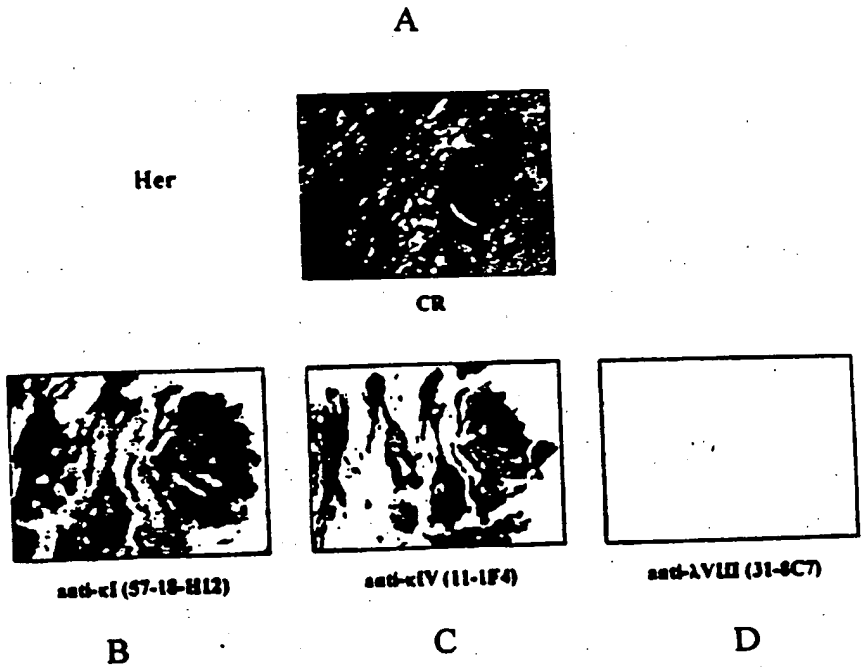

Figure 3

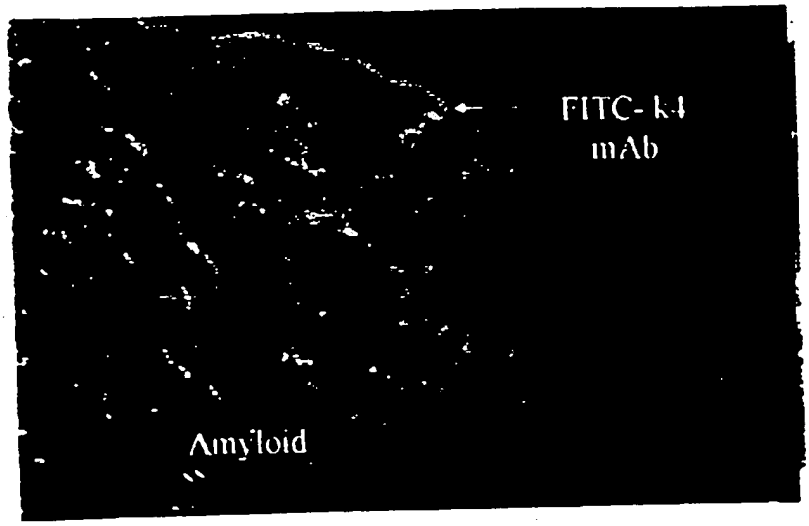

Figure 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/11200

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 16/18, A61K 39/395
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9625435 A1 (BAYER CORPORATION), 22 August 1996 (22.08.96), See page 1, lines 4-7 and lines 19-23; page 6, lines 11-22; page 7, lines 21-24; page 12, lines 19-20	1,4,8,10,11, 18-22
A	--	2,3,5-7,9, 12-17

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
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"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search	Date of mailing of the international search report
31 August 1999	07 10 1999
Name and mailing address of the International Searching Authority European Patent Office P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel(+31-70)340-2040, Tx 31 651 epo nl, Fax(+31-70)340-3016	Authorized officer Carl-Olof Gustafsson/Els

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/11200

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Bioconjugate Chem., Volume 5, 1994, Ulrich Bickel et al, "Development and in Vitro Characterization of a Cationized Monoclonal Antibody against BetaA4 Protein: A Potential Probe for Alzheimer's Diseasel", page 119 - page 125, see abstract and page 124, lines 6-14 (right column)	10-12,15, 20-22
A	--	1-9,13,14, 16-19
A	WO 8901343 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA), 23 February 1989 (23.02.89) --	1-22
A	Journal of Neuropatholy and Experimental Neurology, Volume 53, No 4, July 1994, Lary C. Walker et al, "Labeling of Cerebral Amyloid in Vivo with a Monoclonal Antibody", page 377 - page 383, See abstract -- --------	1-22

INTERNATIONAL SEARCH REPORT

Ir. ational application No.
PCT/US 99/ 11200

Box I Observations where certain claims were found uns archable (C ntinuation f item 1 f first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-9 relate to a method of treatment of the human or animal body by surgery or therapy/diagnostic methods practised on the human or animal body/Rule 39.1(iv), the search has been carried out and based on the alleged effects of the compounds/compositions.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/08/99

International application No.
PCT/US 99/11200

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9625435 A1	22/08/96	CA 2212887 A	22/08/96
		EP 0809656 A	03/12/97
		JP 10509736 T	22/09/98
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